Limbal stem cell deficiency in cats: Etiology, clinical manifestations, diagnosis and management

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ABSTRACT

Limbal stem cell deficiency is a progressive process that causes a severe cellular reaction on the corneal surface and can result in blindness in animals, especially in cats. Many medical and surgical methods have been developed to increase the limbal epithelial stem cell population or for the restoration and reconstruction of the limbal region. With the advancements in science and technology today, cell-based regenerative therapies hold promise for the treatment of limbal stem cell deficiency in animals. This review has been prepared to evaluate the etiology of limbal epithelial stem cell deficiency, to reveal effective diagnostic criteria in determining the disease, and to provide a general perspective on the therapeutic management in cats.

Introduction

The cornea achieves and sustains its transparency through a specialized cellular structure, a fluid-regulated environment, and the protective mechanisms provided by a structure referred to as the limbus, which also actively contributes to corneal regeneration and forms the border with the sclera (13). The epithelial cells composing the primary cellular body of the limbus function as a barrier, preventing the uncontrolled migration of conjunctival epithelium onto the cornea. Concurrently, the limbal epithelial stem cells within its composition play a pivotal role in supporting numerous cellular functions, with a particular emphasis on corneal regeneration (54). The deterioration of the limbal region and, consequently, the impairment of limbal epithelial stem cells disrupt the homeostasis of the cornea, leading to a rapid loss of transparency. This process results in vision impairment in several species. Research has illuminated that Limbal Stem Cell Deficiency (LSCD) constitutes a significant contributing factor to cornea-related blindness, affecting an estimated 45 million people globally (16). Although an exact number of cases in animals remains undetermined, LSCD, arising from various factors, holds the potential to become a prevalent pathological condition. These contributing factors encompass trauma induced by chemical, heat, and radiation burns, genetic diseases, as well as systemic conditions provoking chronic inflammation in the limbal region (53).

In parallel with advancements in human medicine, ongoing efforts are directed in veterinary medicine, toward the development of treatment options aimed at supporting the limbal region and, consequently, the corneal stem cells. This review has been prepared to evaluate the etiology of limbal epithelial stem cell failure, to reveal effective diagnostic criteria in determining the disease, and to provide a general perspective on the therapeutic management of limbal deficiency in cats.

The Structure of Limbus and the Biology of Limbal Stem Cells

The limbus, a layer abundant in blood vessels and cells, serves as a crucial barrier between the cornea and sclera. Distinguished from the cornea by the presence of Langerhans cells and melanocytes, and histologically differing from the conjunctiva by the absence of goblet cells (8), the limbus is characterized by brown radial extensions known as Vogt palisades, housing limbal epithelial stem cells. These specialized structures, identified in humans and pigs, encompass blood vessels, lymphatic nerves, and stem cells (32, 46). Horses and rabbits have crypt-like structures instead of Vogt palisades. Notably, invaginated structures are observed in this region in dogs, sheep, cattle, and mice. Cats and rats, on the other hand, lack Vogt palisades or crypt-like structures in the limbus. While the corneal stem cells are primarily located in the limbus in humans and many animal species, a distinct nomenclature is attributed to them: limbal stem cells. However, studies have identified the presence of stem cells outside the limbus in the cornea of dogs, cattle, sheep, and mice. Consequently, the term "corneal stem cell" is employed in these species instead of "limbal stem cell" (46).

Limbal epithelial stem cells (LESC) are specialized cells crucial for maintaining the integrity of the corneal surface by promoting homeostasis, corneal regeneration, and reepithelialization during wound healing processes (13, 54). Possessing unlimited proliferation capacity, LESCs undergo a perpetual cycle of renewal. Throughout this cycle, stem cells differentiate into distinct cell groups, with LESCs primarily giving rise to transient amplifying cells (TACs) characterized by limited proliferative capacity. TACs infiltrate the basal layers of both the limbal and corneal epithelia. Subsequently, TACs transform into post-mitotic cells (PMCs), which further differentiate into terminally differentiated cells (TDCs). These TDCs progress to the corneal surface, eventually shedding from the corneal surface. This continuous cycle ensures the perpetual renewal of the cornea (8). Moreover, research indicates that mesenchymal stem cells (MSCs) migrate to the inflamed cornea following corneal injuries, particularly after thermal burns. MSCs, originating from the bone marrow under the influence of stromal cell-derived factor-1 (SDF-1) and substance P in the peripheral blood, play a crucial role in damaged corneas. These MSCs arriving at the cornea have demonstrated significant support for corneal epithelium regeneration by enhancing the expression of anti-inflammatory cytokines, including transforming growth factor-β (TGF-β) and interleukin-1Ra (IL-1Ra). Additionally, beneficial soluble factors such as epidermal growth factor (EGF) are known to be secreted for the restructuring of the limbal microenvironment (24, 41).

Limbal Stem Cell Deficiency

Limbal stem cell deficiency (LSCD) is an ocular pathology capable of inducing vision loss, manifesting as a consequence of damage to the limbal stem cells within the limbus and its adjacent regions. The inflicted damage impairs the barrier function that normally exists between the conjunctiva and the cornea, allowing the migration of conjunctival epithelial cells onto the cornea. This migration constitutes a defining characteristic of LSCD. Furthermore, following the deterioration, neovascularization ensues in both the corneal epithelium and stroma, leading to corneal opacity and subsequent vision loss (32). Various acquired, immunological, and genetic factors may contribute to the etiology of LSCD. Additionally, LSCD may arise as a consequence of systemic and immune-mediated diseases, such as viral infections (13, 53).

Etiological Factors and Effects in Limbal Stem Cell Deficiency

Acquired Factors: Acquired factors encompass chemical, thermal, and radioactive burns, drug toxicities, as well as direct eye traumas in animals. Chemical burns, comprising alkaline and acid burns, represent a prevalent type of injuries in cats, constituting a significant aspect of the overall incidence of burns in this population (15). The primary distinction between acidic and alkaline chemicals lies in their impact on the coagulation mechanism of proteins within the epithelial layer of the cornea. The elevated pH of alkaline chemicals prevents the denaturation of surface proteins and avoids inducing coagulation alterations. Consequently, the alkaline agent can penetrate more deeply, leading to additional destruction of the epithelial layer and stroma, resulting in profound burns. Additionally, these agents give rise to non-healing chronic ulcers as they swiftly obliterate all cells they encounter, including the stem cells of the corneal epithelium (7, 55). Also, in experimental studies involving animal models designed to explore the impact of alkaline agents on the eye, the pronounced destructive effects of these agents on the cornea and limbal stem cells are distinctly evident. This phenomenon has led to the observation of LSCD in both human and animal models, as documented by Kethiri in 2019 in a medical journal. While injuries to the limbus resulting from ocular trauma have been documented (51), no study on limbal stem cell failure has been identified in the international literature.

Immunological factors: Feline Herpesvirus-1 (FHV-1), identified as one of the viruses impacting the limbal region, stands out as a predominant viral cause of ocular surface infections (33). Serological studies indicate a

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widespread prevalence of FHV-1 in the global cat population, with reported exposure rates reaching as high as 97%. Following infection, the virus initially targets the nasal mucosa and conjunctiva, establishing residence in the epithelial cells of these areas. Subsequent proliferation leads to significant destruction, particularly in the corneal epithelial cells. This destruction is characterized by acute cellular damage, precipitating rapid viral replication and cytolysis (22, 42). The presence of corneal ulcers is considered a pathognomonic finding of FHV-1 infection (60, 61) (Figure 1).

In humans, the herpes simplex virus has been observed to cause the destruction of stem cells in the limbal region, leading to limbal stem cell deficiency. Examination of human studies has revealed that the lesions induced by the herpes simplex virus in humans closely resemble those observed in cats (38, 44).

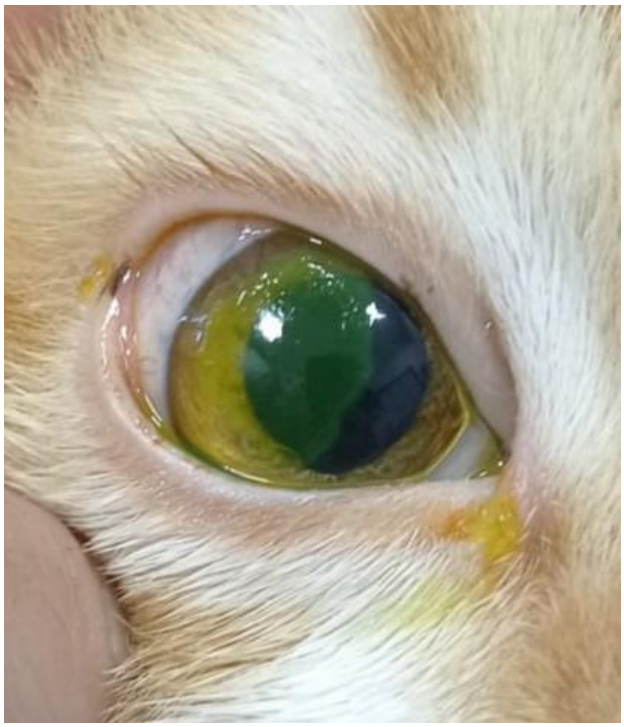

Figure 1. Fluorescein-positive image of a persistent corneal ulcer due to herpes virus in a 7-month-old tabby cat.

Clinical Manifestations of Limbal Stem Cell Deficiency

The alteration and degradation in the limbus disrupt the integrity of the barrier between the cornea and sclera, leading to the corneal surface being covered by conjunctival epithelial cells. This phenomenon is termed conjunctivalization, representing a key distinguishing feature of LSCD (12, 54) (Figure 2). Clinical symptoms of LSCD can vary based on the severity and extent of damage in the limbal region. In mild cases, a dull and irregular corneal surface, along with epithelial opacity, is observed. This opacity may be attributed to conjunctival epithelial cells lacking neovascularization, adhering to the corneal surface. In moderate cases, there is an increase in neovascularization on the corneal surface, and occasionally, pannus formation may occur. On the corneal surface, an abnormal epithelial structure is evident, and this epithelium is consistently susceptible to erosion. In more advanced cases, recurrent and enduring epithelial defects become frequent due to a reduction in the functional limbal stem cell population. Chronic non-healing corneal epithelial defects and delayed healing subsequent to recurrent epithelial destruction are common clinical symptoms of LSCD (27, 32). In severe cases where the conjunctiva heavily adheres to the cornea, symptoms such as lagophthalmos, obstruction of lacrimal punctums, and dry eyes are also encountered (22). Conjunctival adhesions at different levels and shapes can be observed in severe cases. Eyelid and conjunctival deformations such as ankyloblepharon and symblepharon, may occur as a result of the conjunctival adhesions that form (58) (Figure 3).

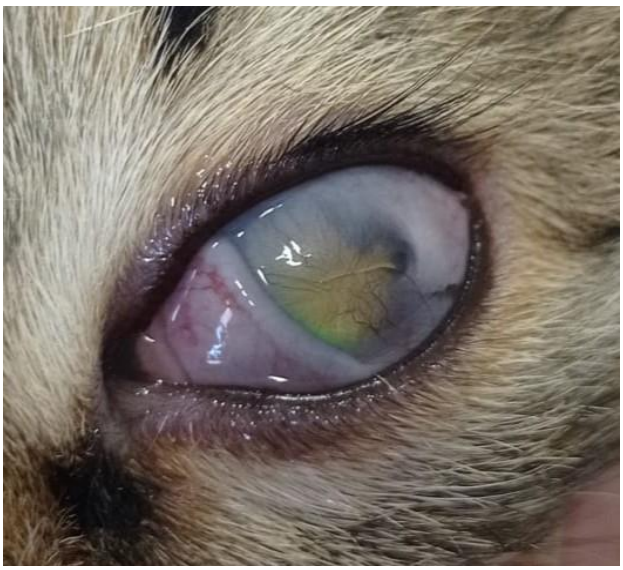

Figure 2. Severe conjunctivalization in a 5-month-old tabby cat. It is noteworthy that the limbus disappears completely, conjunctivalization progresses towards the center in the entire dorsolateral quadrant of the cornea, and the presence of superficial vascularization covering the entire surface of the cornea.

Figure 3. 6-month-old British Shorthair cat with severe conjunctivalization and symblepharon (left). A 1-year-old domestic shorthair cat with ankyloblepharon and symblepharon. The third eyelid adhesion to the corneal surface. Ankyloblepharon is seen at the lateral canthus of the upper eyelid (right).

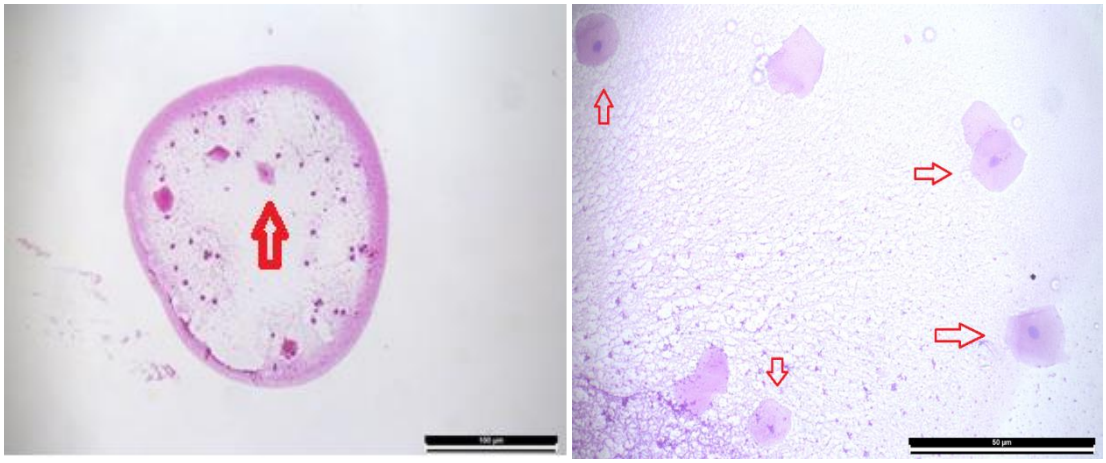

Figure 4. Impression cytology obtained from the corneal surface with conjunctivalization. Image of goblet cell (arrow) with Hematoxylin and eosin (H&E) stain (left). Image of goblet cells (arrows) with Periodic acid-Schiff (PAS) stain (right).

Definitive Diagnosis

In the past, the evaluation of LSCD primarily focused on corneal transparency and other clinical symptoms. Nowadays, various methods that offer more precise and objective data have been incorporated into this assessment. Understanding the cat's medical history is crucial for determining the etiology. Slit lamp biomicroscope examinations are commonly employed in clinical examinations for diagnosis (32). These examinations can be conducted under direct yellow light without fluorescein dye or under cobalt blue light with fluorescein dye. During examinations under direct yellow light, anatomical defects on the corneal surface and limbal region are observed. The thickness and transparency of the corneal surface are assessed, and the presence of conjunctival epithelial cells and neovascularization on the corneal surface is examined. Noticeable loss of detail is observed in the limbal region. In slit lamp examinations performed with fluorescein staining, the presence and distribution of abnormal cells on the corneal surface are visualized due to their affinity for the dye. Since the proportion of conjunctival tissue covering the cornea changes based on the severity of LSCD, alterations in the density of dye adhered to the corneal surface can be observed (27, 32).

The impression cytology (IC) technique stands out as another diagnostic method employed for the assessment of LSCD. In this method, cell samples obtained from the corneal surface are stained with cell dyes using specialized cellulose acetate filter papers. The identification of goblet cells on the corneal surface through IC offers crucial insights for the diagnosis of LSCD, complemented by slit lamp biomicroscopy findings (63). Various apparatus, including small plastic tubes, have been developed to facilitate the collection of IC samples from the cornea in feline subjects (17, 47). It is important to note that the presence of goblet cells on the cornea may not always be conclusive for diagnosing LSCD, as certain pathologies also exhibit this characteristic (Figure 4). This realization has underscored the necessity to explore additional

parameters that can provide more precise evidence of conjunctival tissue presence on the corneal surface.

Today, while intracellular antigen studies continue at full speed, focus is especially on Cytokeratins (K) found in the intracytoplasmic cytoskeleton of epithelial cells. In human field studies, it has been possible to detect LSCD at an early stage by detecting conjunctival cytokeratins on the corneal surface and evaluating the density levels of corneal cytokeratins (36). K3 and K12 cytokines are found in corneal epithelial cells. While K3 is found in all corneal epithelial cells and the suprabasal layer of conjunctival epithelial cells; K12 is found in all corneal epithelial cells and in the suprabasal layer of limbal epithelial cells. K12 is utilized as a corneal-specific cytokeratin. While K13 was detected in the conjunctival epithelium and the suprabasal layer of limbal epithelial cells, K19 was detected in the basal cells of the epithelial layer in the corneal periphery. When the research results are evaluated, the detection of Limbal Stem Cell Deficiency (LSCD) can be demonstrated simply and reliably by immunohistochemical staining for the conjunctiva-specific K13/K19 pair in intracytoplasmic samples. Assessments regarding the severity of the disease can be made by examining the density of corneal-specific K12 (2, 48).

Management of Limbal Stem Cell Deficiency

The first step in LSCD treatment includes discontinuing medications that have been used for an extended period, eliminating irritants to which the individual has been exposed, and addressing any systemic diseases that may be triggering the condition (64). The second step focuses on ensuring corneal transparency and maintaining its continuity. Steroids are commonly recommended to medically suppress inflammatory cells on the ocular surface, and protective eye lubricants are advised to prevent further loss of limbal stem cells within the limbus (16). The third step is centered around various strategies to enhance the limbal stem cell population, facilitating the reconstruction and restoration of the affected region (64).

Methods Used to Increase the LESC Population

1. Limbal transplantation

Conjunctival limbal autografts: Conjunctival limbal autografts obtained from the unaffected eye are directly transplanted into the eye experiencing LSCD. This method is considered reliable due to the utilization of the individual's own tissue. However, a disadvantage exists in terms of the potential risk of inducing iatrogenic LSCD in the healthy eye serving as the donor (12). This method was experimentally applied to dogs by Brunelli et al. and to rabbits by Dios et al. As a result of these studies, corneal transparency was successfully achieved in eyes with LSCD without any complications (8, 14). No published studies have been identified regarding the application of this method to cats.

Simple limbal epithelial transplantation: A small piece of limbal tissue taken from the healthy eye is divided into smaller pieces and transplanted to the recipient's eye together with human amniotic membrane tissue. The lower transparency of the amniotic membrane compared to the cornea and the risk of infection are the disadvantages of this method (6, 12). No published studies have been identified regarding the application of this method to cats.

Allogeneic limbal grafts: This method was developed for animals with LSCD in both eyes that are not suitable for autograft. It involves transplanting limbal tissue obtained from the healthy eye of another organism of the same species. However, this method has significant disadvantages such as the risk of rejection of the transplanted tissue, the requirement for strong immunosuppression, and the potential for the transplanted tissue to carry infectious diseases. There is also a risk of triggering iatrogenic LSCD in the donor's healthy eye (64). The method has been experimentally applied to rabbits and mice (14, 35). No published studies have been identified regarding the utilization of this method in cats.

2. Cultured limbal epithelial cell transplantation: This method is designed to minimize the potential complications associated with the donor eye in limbal transplantation. In this approach, a small piece of limbal tissue is extracted from the donor eye, cultured, and propagated in an ex-vivo environment. The resulting cells are then transplanted into the eye with LSCD using human amniotic membrane (56). No published studies have been identified regarding the application of this method in animals.

3. Non-limbal epithelial cell transplantation: It is an alternative method developed in line with the disadvantages of other methods and also by evaluating the need for autologous epithelial cells. The most commonly used autologous cells in this method are oral mucosa epithelial cells, conjunctival epithelial cells, and epithelial-like cells differentiated from pluripotent or multipotent stem cells. While the use of oral mucosa epithelial cells provided an average of 72% success in terms of surface stabilization, conjunctival epithelial cells provided an 86% success rate. However, since the transplanted tissue phenotype was different, the quality of vision was less than optimal. The use of corneal epithelial cells derived from pluripotent or multipotent stem cells is still under investigation; However, the biggest ethical

concern in these methods is the risk of triggering tumor formation (25, 64). No published studies have been identified regarding the application of this method in animals.

Methods Used for Limbal Region Restoration and Reconstruction

1. Bioactive extracellular matrix (ECM): ECM is a very important component for the function of the cells in the limbal area. For this reason, especially current LSCD treatment options are evaluated with a focus on the continuity of ECM function.

Amniotic Membrane: Amniotic membrane is currently a very popular scaffold for ocular surface reconstruction. Among LSCD treatment options, it is preferred to provide ECM support to the region. The ECM inherent in the amniotic membrane contains collagen types I, III, IV and V, laminin 1 and 5, fibronectin and various growth factors/cytokines such as epidermal growth factor and hepatocyte growth factor. Thanks to the dense collagen fibers in its structure, it provides a solid settlement for the cells. In this way, it has been suggested that the amniotic membrane can provide a suitable environment for LESCs in the limbal area (45). When the uses of amniotic membrane transplantation on animals are examined, it has been used in ocular surface restoration in horses and in cats to support the cornea after corneal necrosis surgery, and appropriate and positive results have been obtained in the healing of the corneal surface (4, 49). In addition, the use of human amniotic membrane has been used after corneal necrosis surgery in cats and in corneal wounds in dogs, and positive results have been obtained in corneal healing (26, 30).

However, in addition to the positive features listed above, the amniotic membrane also has some negative features. This membrane is an opaque tissue with low tensile strength and at the same time, since it is a biological material, there is a risk of carrying infectious diseases (50). Additionally, since the amniotic membrane is digested after transplantation, the ECM support it provides to the limbal region is limited in the long term (29).

Fabricated Bioactive ECM: Studies have shown that the production of bioactive ECM may be a potential strategy that can be used to restore the function of the limbal region. Protocols used to produce bioactive ECM generally rely on the use of purified/recombinant structural proteins such as collagen or decellularization of animal or human corneas. It has been observed that bioengineering products obtained by using structural proteins ensure the alignment and proper structure of the limbal region, as well as enable the proliferation and phenotype of human LESCs. Decellularization of porcine and human corneas has been studied as another approach to produce a bioactive ECM. The functions of decellularized corneas, their bioactivity, and the placement of corneal epithelial cells on the prepared scaffolds have been demonstrated by transplantation in animal models. In addition, decellularized pig cornea was used in cases of corneal ulceration as a clinical study and its results were investigated. According to the results, this approach is mostly applicable in cases that require healthy epithelium and stromal replacement, and therefore its application in LSCD may be limited. Therefore, an alternative protocol for the production of bioactive ECM that can be used in LSCD has been proposed by digesting decellularized corneas and producing a hydrogel. It was observed that this hydrogel provided suitable support for in vitro culture of corneal stromal cells. Thus, it appears that the production of a bioactive ECM hydrogel from decellularized corneas may be a potentially effective strategy for reconstructing the limbal region. The main goal in this strategy is to create a bioactive ECM that contains healing factors as well as structural proteins (11, 64).

2. Biological factors: As stated in other treatment options, the continuity of the function of the limbal region largely depends on proper communication and signaling between cellular components. While most of the critical signaling factors continue to be identified after damage to the region, local distribution of exogenous growth factors to the region is a remarkable approach to restore function in the region.

Hemoderived factors: Serum or plasma produced from blood can be used as eye drops. This is a method that can be encountered quite frequently in routine clinical practice. Eye drops obtained from serum and plasma contain tear-like growth factors, cytokines, vitamins and minerals. Thereby, they support corneal epithelial homeostasis, proliferation and differentiation. Due to these effects, autologous/allogeneic serum eye drops and platelet-derived preparations are among the options used to restore limbal region function (28).

It has been proven that eye drops prepared with autologous serum contain many factors necessary for the homeostasis of the limbal and corneal epithelium (3). In clinical studies, successful rehabilitation of the ocular surface was observed after the application of autologous serum to patients with corneal epithelial defects. It has been observed that it provides a healthy ocular surface restoration, especially in patients with permanent epithelial defects due to LSCD (21, 62).

Another blood-derived factor used for the eye is obtained from platelets. They are potentially useful in

regenerating the limbal region through the influence of growth factors within platelets (20, 28). In a clinical study conducted by Farghali et al., the healing process of corneal ulcers in cats and dogs, both deep and superficial, was meticulously observed subsequent to the subconjunctival administration of autologous platelet-rich plasma (PRP). The findings suggest that the application of PRP significantly accelerates corneal healing at a noteworthy rate, establishing it as a cost-effective and easily implementable method (19).

Bio-active soluble factors: Bioactive soluble factors are biological factors obtained by purification from living tissues, cell secretomes and/or through recombinant techniques. These soluble factors, available from various sources, have been investigated for the regeneration of the ocular surface and limbal region (18). *Amniotic membrane* extract eye drops were obtained for this purpose. It is obtained by homogenizing and centrifuging human amniotic membrane tissue and then collecting the supernatants. This structure obtained is a mixture of soluble factors of the amniotic membrane, and in vivo studies have shown that it increases the cultivation of limbal stem cells in patients with LSCD (5). In an experimental investigation, the application of amniotic membrane extract eye drops was observed to facilitate the in vitro proliferation of limbal stem cells and induce corneal healing in rabbits afflicted with corneal ulcers, exhibiting no adverse effects (57). Conversely, an experimental study conducted on horses by Lyons et al. in 2021 revealed that the utilization of amniotic membrane extract did not yield significant healing outcomes in the control group with experimentally induced corneal ulcers (39).

Another soluble factor used for ocular surface regeneration is *pigment epithelial derived factor (PEDF)*, a growth factor extracted from blood plasma. In vitro studies have shown that PEDF and its derivatives support the self-regeneration of limbal epithelial stem cells and increase their proliferation rate. At the same time, in vivo studies conducted on animal models with corneal epithelial damage have shown that PEDF provides restoration of the limbal region (23). In experimental investigations conducted on mice, it has been established that PEDF plays anti-inflammatory and immune regulatory roles. Furthermore, the findings of these studies indicate that PEDF exerts a regulatory effect on the ocular surface (40, 59).

Secretomes, which contain all the factors released by in vitro cultured cells, are another factor used for ocular surface restoration. In particular, the secretome of mesenchymal stem cells is a combination of factors that are very useful and healing in the revitalization of the limbal region and ocular surface (18).

Another one of the soluble factors is *extracellular vesicles*. Cells carry out their communication and functions among themselves through the extracellular vesicles they secrete. Exosomes, one of the smallest of the extracellular vesicles, have proven to have powerful therapeutic effects. They contain various nucleic acid types and derivatives, lipids and proteins. Since exosomes have very good biodistribution, biocompatibility and low immunogenicity, they can be safely used therapeutically (10, 24). It is recognized that exosomes are absorbed by corneal epithelial cells both in vitro and in vivo. These exosomes exhibit a beneficial presence in reconstruction of the limbal region and ocular surface in animal models. These outcomes have significantly enhanced the utilization of mesenchymal stem cell-derived exosomes, especially in the restoration of the ocular surface and limbal region (10, 52).

3. Cell-based approaches: Stem cells are one of the most commonly used therapeutic agents today. Mesenchymal stem cells (MSCs) have played an important role in the reconstruction of the ocular surface and limbal region, especially in recent years. Many studies have been conducted in animal models on the use of MSCs obtained from various sources in various ocular surface disorders, including chemical burns, dry eye syndrome, LSCD, and corneal transplantation. In these studies, the reconstruction provided by MSCs on the corneal surface was examined (9, 34). Studies have been conducted on limbal mesenchymal stem cells (L-MSC) and when the results are evaluated, significant developments have been made. L-MSCs are in close contact with limbal epithelial stem cells and have an important role in protecting the limbal region. In addition, it has been observed in studies that L-MSCs have very similar properties and gene expression patterns to MSCs derived from bone marrow. Moreover, similar to other MSCs, L-MSCs have immunomodulatory properties and inhibit immune cells, including T cells, in vitro. In rat alkali burn cornea animal model experiments conducted upon detection of all these effects, it was observed that topical or subconjunctival applications of L-MSCs provided a decrease in corneal opacification, a regression in neovascularization and an improvement in fluorescein staining results (1). In line with all these researches and studies, it seems very likely that MSCs support the restoration of the limbal region by secreting regenerative factors and thus can be used in LSCD treatments.

MSCs secrete biodegradable factors known as extracellular vesicles, as previously discussed (24). Numerous studies have demonstrated that these vesicles, derived from MSCs, exhibit nearly identical functions to MSCs. Simultaneously, therapeutic applications of

exosomes, the smallest among these vesicles, have been shown to offer advantages over the direct use of cells (37, 41). Exosomes boast several favorable attributes, including a low risk of immune reactions, tumor formation, and infection. Moreover, by utilizing exosomes instead of cells, the potential transfer of mutated or damaged DNA is eliminated. Additionally, owing to their diminutive size, exosomes can effectively traverse ocular barriers, including the tear film barrier, conjunctival, vitreal, corneal barrier, as well as blood-humor aqueous and blood-retina barriers in the eye (31, 43, 65). The authors are currently undertaking projects related to the utilization of exosomes in limbal epithelial regeneration and the treatment of LSCD in cats.

Conclusion

Limbal stem cell deficiency (LSCD) is a progressive process, particularly in cats, which induces severe cellular reactions on the corneal surface, disrupting corneal homeostasis and potentially resulting in blindness. Numerous exogenous and endogenous factors, primarily herpesvirus, capable of damaging the limbal region, can lead to LSCD. Many LSCD treatment options have been created in both human and veterinary fields to increase the number of Limbal stem cells and ensure the restoration of this region. These protocols, each offering distinct advantages, are continually evolving with advancements in current technology. Processes wherein the treatment primarily targets the stem cell production capacity of the limbus have propelled researchers towards cell-based approaches. This has led to an increased focus on the study of stem cells and extracellular vesicles, such as exosomes. With their significant role in regeneration processes, it is inevitable that LSCD will be recognized as the sole and definitive treatment option in the future. It is believed that the favorable outcomes achieved in the field of human ophthalmology will offer promise for animal research. Also, the authors are currently conducting a research project, which explores the potential utilization of allogeneic mesenchymal stem cell-derived exosomes for treating LSCD in cats with conjunctivalization resulting from limbal insufficiency.

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Ethical Statement

This study does not present any ethical concerns.

Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

The authors confirm a group work for interpretation and preparation of the manuscript.

Data Availability Statement

Data available on request from the authors.

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